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Phytochemical Screening and Antimicrobial Potential of *Barteria nigritana* Leaf Extracts in Different Solvents against *Staphylococcus aureus* and *Escherichia coli*

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ABSTRACT:
The present study investigated the phytochemical screening and antimicrobial potential of methanolic, ethanolic and water extracts of the leaves of *Barteria nigritana* against clinical isolates (*staphylococcus aureus* and *Escherichia coli*). The phytochemical screening of the leaves of *B. nigritana* was carried out for the estimation of saponins, tannins, flavonoids, cardiac glycosides, deoxysugars, terpenes, phlobatannins, anthraquinones, alkaloids, phenols and carbohydrates. The antibacterial assay was carried out using the disc diffusion method. The phytochemical screening showed the presence of saponins, flavonoids, cardiac glycosides, deoxysugars, terpenes, alkaloids, and carbohydrates but absence of tannins, phlobatannins, anthraquinones and phenols. The antimicrobial assay showed some level of reactivity of all the extracts against the growth of *Escherichia coli* (gram negative bacteria) at an inhibition zone of 8mm in diameter, while *Staphylococcus aureus* (gram positive bacteria) was found to be resistant to all the extracts.
Keywords: *Barteria nigritana, Escherichia coli, Phytochemicals, Staphylococcus aureus*

INTRODUCTION:

A medicinal plant is any plant in which one or more of its organ contains substances that can be used for therapeutic purposes on which are precursors for the synthesis of useful drugs. Medicinal plants contain biologically active chemical substances (phytochemicals) such as saponins, tannins, essential oils, flavonoids, alkaloids and other chemical compounds, which have preventive and curative properties. These complex chemical substances of different compositions are found as secondary plant metabolites in one or more of these plants and are useful for humanity [1]. Historically, medicinal plants have been used since ancient times. Medicinal plants typically have essential oils in their tissues or seeds that prevent bacteria, molds, or other microbes from growing. This quality confers antimicrobial properties. Medicinal action of plants is referred to some important chemical compounds that pass on a definite physiological action on the human body [2].

The frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality in immunocompromised patients in developing countries [3]. The increasing prevalence of multi-drug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raised the specter of 'untreatable' bacterial infections and adds urgency to the search for new infection-fighting strategies [4]. For a long time, plants have been an important source of natural products for human health. The antimicrobial properties of plants have been investigated by a number of studies worldwide and many of them have been used as therapeutic alternatives because of their antimicrobial properties [5]. Despite the existence of potent antibiotic and antifungal agents, resistant or multi-resistant strains are continuously appearing, imposing the need for a permanent search and development of new drugs [6]. Plants are considered the cheapest and safer alternative sources of antimicrobials.

Barteria comprises of four species, which all occur in tropical Africa. *Barteria nigritana* is a Shrub or a tree of ± 10m (outside East Africa up to 25m). It is sometimes deciduous with the stem up to 30–50cm in diameter and the bark whitish and rough. Leaf-blades are variable in shape. They range from elliptic to oblong or ovate-oblong, or oblanceolate, base rounded to long-cuneate (especially in terminal leaves), top ± obtuse to acute, or acuminate up to 2 cm, [10–30(–45)] by [4–10(–19)]cm, subcoriaceous, glabrescent or glabrous; nerves 9–19 pairs, ± prominent on both sides.The petiole is 5–10(–15) mm long, 2–8 mm. wide, mostly shortly winged and decurrent on the branchlets as a low ridge. Inflorescences are 1–4-flowered (or outside E. Africa up to 9-flowered). The bracts are orbicular to ovate, obtuse or shortly acuminate, 3–15 mm long, outside ferrugineous pubescent or glabrous, margin ciliate. Flowers are large and whitish. Sepals ranges from elliptic to oblong, acuminate (2.5–4 by 1–1.5 cm), ferrugineous silky or glabrescent outside. Petals resembling the sepals, equal in size or either somewhat smaller or larger, mucronate. The Outer corona is membranous (± 1 cm high) while the inner corona is fleshy and shallowly lobed (3–5 mm high). The Stamens is ± 3 cm. The filaments connate for nearly half-way and it is glabrous with anthers (3–6 mm). The ovary is glabrous, sub-globose or with 3–5 bulges on the upper part. The style is 10–20 mm with stigma ranging from conical to subglobose in shape and yellow in colour. Fruit is green-yellow to dull orange or reddish and it is ± 1.5–3 cm in diameter.

Barteria nigritana through a previous study has been shown to be in a symbiotic mutualism with a community of ants [7]. In Cameroon, the Baka people use *Barteria*

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nigritana to treat anaemia and toothache and in Congo, the Kouilou and Mayombe people use the bark to treat wounds, scabies and itch. After a wash with the decoction, the affected area is dusted with powdered bark [8]. Hence, the present study was undertaken specifically to investigate the role of aqueous, methanol and ethanol leaf extracts of *Barteria nigritana* as a potential antimicrobial agent against some human pathogenic bacteria.

MATERIALS AND METHODS

1. Plant Preparation and Extraction

Freshly collected leaves of *Barteria nigritana* were rinsed under running tap water, cut into smaller sizes and air dried for two weeks. They leaves were pulverized, stored and preserved in a sealed container prior to extraction. The sample (500g) was macerated with 95% Ethanol, Methanol and distilled water and left for 72 hours for complete extraction. The extract was then filtered into a clean beaker using funnel and cotton wool. The filtrate obtained was evaporated to dryness using rotary evaporator and water bath to obtain the dry extract at a low temperature. The dry extracts were concentrated to various concentrations of 128, 64, 32, 16, 8, 4, 2 and 1 mg mL^{-1}. The extracts were stored at 4°C in airtight bottles.

2. Phytochemical Analysis on the Extract

Phytochemical analysis of the plant components was carried out using standard method of chemical analysis for the presence of saponins, tannins, flavonoids, cardiac glycosides, deoxysugar, terpenes, phlobatannins, anthraquinones, alkaloids, phenols and carbohydrates in the samples.

2.1 Test for presence of Saponins:

The plant extract 0.5g was stirred with 5ml of distilled water and shaken vigorously(for frothing test).0.5g of plant extract and 5% $Na_2CO_3$was mixed and drops of Fehling's solution was added and boiled. It was observed for the presence of a stable brown-red precipitate [9].

2.2 Test for presence of tannins:

The plant extract 0.25g was stirred with 5ml of distilled water and filtered. 5% ferric chloride reagent was added to the filtrate and observed for a dark green colouration [10].

2.3 Test for presence of Flavonoids:

A small quantity of the extract was dissolved in 2ml of distilled water and warmed. 4 drops of concentrated hydrochloric acid (HCl) followed by addition of few pieces of magnesium metal. The appearance of orange color was observed for the presence of Flavonoids [10]

2.4 Test for presence of Cardiac Glycosides:

0.5g of the plant extract was dissolved in 2ml of Chloroform. Concentrated H_2SO_4 was added carefully to form a lower layer. A reddish brown colour was observed at the interphase for the presence of cardiac glycoside [11].

The main aim of this study is to to investigate the phytochemical components present in *Barteria nigritana* and to determine the antimicrobial activity of the leaves of *Barteria nigritana* in different solvents *(*methanol, ethanol and water*)* against some selected clinical isolates in view of their use as alternative sources of antimicrobial drugs used in the treatment of diseases.

2.5 Test for presence of DeoxySugar:

0.5g of the plant extract was dissolved in 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlaid with 1ml of concentracted H_2SO_4.A violet ring observed at the interface indicate positive test [11]

2.6 Test for presence of Terpenes:

3ml of chloroform was added to0.5g of the plant extrac tand filtered, 10 drops of acetic anhydride was added to the filtrate. 2 drops of concentrated Sulphuric acid was added. A pink colour at the interphase indicated the presence of terpenes [11].

2.7 Test for presence of Phlobatannins:

6 drops of dilute HCl is added to a small quantity of the plant extract and heated to boiling point. Deposition of a red precipitate formed is allowed to cool. The precipitate was washed with hot water. A bulky precipitate after washing indicates the presence of phlobatannins.

2.8 Test for presence of Anthraquinones:

The plant extract of 0.25g was boiled with 5ml of 10% H_2SO_4 and filtered. The filtrate was shaken with 2.5ml benzene. The benzene layer separated and 5ml of 10% NH_4OH was added. A pink or violet colour in Ammonia phase (lower phase) indicates the presence of anthroquinones [11].

2.9 Test for presence of Alkaloids:

15mls of 10% HCL was added to 0.5g of sample and boiled for 10minutes. The sample was filtered, allowed to cool and divided into 3 test tubes. To the first test tube, few drops of dragendroff's reagent was added and formation of red precipitate indicated the presence of alkaloid. To the second test tube, Hager's reagent was added and formation of yellow coloured precipitate indicates the presence of alkaloid. Mayer's reagent was added and formation of a white or cream precipitate indicates the presence of alkaloids [10].

2.10 Test for presence of Phenols:

0.5g of extract was treated with 4 drops of ferric chloride solution. Appearance of blue colour indicates the presence of phenols [11].

2.11 Test for presence of Carbohydrates:

The extract was dissolved in 5ml distilled water and filtered. The filtrate was treated with 2 drops of alcoholic α-napthol solution in the test tube. Formation of violet

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ring at the junction indicates the presence of carbohydrates (molisch's test [11].

3. Antimicrobial Assay

Varying concentrations (128, 64, 32, 16, 8, 4, 2 and 1 mg mL^{-1}) of the methanolic, ethanolic and aqueous leaf extracts of *Barteria nigritana* was prepared and used to assay for antibacterial activity on representative Gram positive and negative bacterial isolates. Clinical samples of *Escherichia coli*(Gram negative) and *Staphylococcusaureus* (Gram positive) were obtained from the University of Uyo Teaching hospital, Uyo. The test isolates were subcultured and maintained on brilliance E.coli/coliform medium (Oxoid) and mannitol salt agar (Oxoid) respectively, and comparatively assayed for susceptibility to the plant extract using disc diffusion method (Kirby-Bauer) according to the NCCLS recommended guidelines.

Colonies of test isolates from each plate were separately transferred into test tubes containing 5 mL nutrient broth and incubated at 36 ± 2°Cfor 24 hours to reactivate the cells. The reactivated organisms were inoculated unto 5 mL of sterile phosphate buffered saline (PBS) and the optical density adjusted to 0.5 MacFarland standard (containing approximately 1 x 10^8 CFU mL^{-1}). 25 - 30mL freshly prepared and cooled Mueller-Hinton agar (MHA) in duplicate petri dishes were allowed to set. The plates were seeded with 1mL of the test bacterial suspension in PBS, swirled to evenly distribute the inoculum and kept on the bench for 1 hour to dry. Sterile forceps was used to place the filter paper soaked with varying concentrations (128, 64, 32, 16, 8, 4, 2 and 1 mg mL^{-1}) of the different plant extract on the seeded agar plates and gently pressed onto the agar surface to ensure firm contact. Phenicol (Chloramphenicol, C, 30 µg) and oxacilin (OX, 1 µg) commercial antibiotic were used as control.

The inoculated plates were allowed to stand for an hour on the bench for pre-diffusion and incubated at 36± 2 °C for 24 hours. On the basis of the diameter of clear zone around the paper disc, the degree of resistance or susceptibility was determined. The resulting zones of bacterial inhibition were measured using vernier caliper to the nearest whole millimeter. The average of duplicate determinations was taken as inhibition zones of the bacterial growth for a particular concentration of the plant extract.

RESULTS

The results of the evaluation of the presence of phytochemicals in the leaves of *Barteria nigritana* is summarized in Table 1. The phytochemical screening revealed the presence of saponins, flavonoids, deoxysugar, terpenes, alkaloids and Carbohydrates and absence of tannins, phlobatannins, anthraquinones and phenols in leaf extracts of *Barteria nigritana.*

The results of the antimicrobial activity of methanol, ethanol and water extracts of leaves of *Barteria nigritana* against clinical isolates of *Escherichia coli* and *Staphylococcus aureus* is summarized in Table 2, 3 and 4. The results showed that the methanolic, water and ethanolic leaf extracts exhibited some level of susceptibility against the gram negative organism *(Escherichia coli)* and resistance against the gram positive *(Staphylococcus aureus)* as seen in Table 5 which was only susceptible with the standard antibiotic (Oxacilin) used as control.

Table 1: Phytochemical Test Availability of leaf extracts of *Barteria nigritana*

PHYTOCHEMICAL COMPONENTS/ TESTS	EXTRACTS		
	Methanol	**Ethanol**	**Water**
Saponins			
a) Frothing test	**+**	**+**	**+++**
b) Foam test	**+**	**+**	**+**
Tannins	–	–	–
Flavonoids	+	++	+
Cardiac Glycosides	++	+++	+
Deoxysugar	+	+	+++

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Terpenes	+++	+++	+
Phlobatannins	–	–	–
Anthraquinones	–	–	–
Alkaloids			
a) Dragendroff's test	+	++	++
b) Hager's test	++	+	++
c) Mayer's test	+	++	+
Phenols	–	–	–
Carbohydrates	++	++	+

Key: **+** = Present in trace concentration
++ = Present in moderate concentration
+++ = Present in high concentration
- = Absent

Table 2: Zones of Inhibition of Methanolic Leaf Extract of *Barteria nigritana* on Test Organisms

Test Organisms	**Concentration of Extract (mg ml^{-1})/ Inhibition Zones (mm)**							
	128	**64**	**32**	**16**	**8**	**4**	**2**	**1**
Staphylococcus aureus	-	-	-	-	-	-	-	-
Escherichia coli	8mm	8mm	8mm	8mm	8mm	8mm	8mm	8mm

Table 3: Zones of Inhibition of Ethanolic Leaf Extract of *Barteria nigritana* on Test Organisms

Test Organisms	**Concentration of Extract (mg/ml)/ Inhibition Zones (mm)**							
	128	**64**	**32**	**16**	**8**	**4**	**2**	**1**
Staphylococcus aureus	-	-	-	-	-	-	-	-
Escherichia coli	8mm	8mm	8mm	8mm	8mm	8mm	8mm	8mm

Table 4: Zones of Inhibition of Aqueous Leaf Extract of *Barteria nigritana* on Test Organisms

Test Organisms	**Concentration of Extract (mg/ml)/ Inhibition Zones (mm)**							
	128	**64**	**32**	**16**	**8**	**4**	**2**	**1**
Staphylococcus aureus	-	-	-	-	-	-	-	-
Escherichia coli	8mm	8mm	8mm	8mm	8mm	8mm	8mm	8mm

Table 5: Zones of inhibition of Commercial Antibiotic (Oxacilin) on Test Organisms

Test Organisms	**Oxacilin (1 µg)/Inhibition Zones (mm)**
Staphylococcus aureus	10mm
Escherichia coli	8mm

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DISCUSSION

The ability of the extracts to inhibit the growth of the microorganisms have been reported to be as a result of the presence of phytochemicals (alkaloids, glycosides, flavonoids, phenols, saponin, terpenes, tannin) in their leaves which has been seen in recent studies [12], [13]. Saponins, flavonoids, Cardiac glycosides, deoxysugars, terpenes, alkaloids and carbohydrates were present in the methanolic, ethanolic and water extracts. Water extract showed high concentration of saponins and deoxysugars with moderate concentration of terpenes and alkaloids when compared to the methanolic and ethanolic extract. Cardiac glycosides were present in high concentration in ethanolic extract and terpenes was also present in high concentration in methanolic and ethanolic extract. Presence of terpenes in high concentration explains the symbiote characteristic of the family Passifloraceae which is in line with de Almeida *et al*.[14].

The screening showed the absence of tannins, phlobatannins, anthraquinones and phenols in all the extracts. Anthraquinones have been reported for its anticancer properties [15].Research reports also show that phenolic compounds carry out potent antioxidant activity and wide range of pharmacologic activities which include anti-cancer, antioxidant and platelet aggregation inhibition activity [16].Tannins have been reported to inhibit pathogenic fungi [17]. Phlobatannins have also been reported for its wound healing properties, these are anti-inflammatory and analgesic [18] and antioxidant [19]. The absence of tannins, phlobatannins, anthraquinones and phenols in *Barteria nigritana* leaves reveals that the plant may not be effective as anticancer, anti-inflammatory, antioxidant, and analgesic. The absence of anthraquinones and the presence of carbohydrates, glycosides, flavonoids and alkaloids show similar result to previous studies by Razia *et al*., [20] on *Passiflora edulis* in the family Passifloraceae.

Escherichia coli showed some level of sensitivity to the aqueous, methanolic, and ethanolic leaf extracts of *Barteria nigritana* with inhibition zone of 8mm, while *Staphylococcus aureus* was reavealed to be resistant to all the extract. The commercial antibiotic, Oxacilin (1 µg), inhibited the growth of *Staphylococcus aureus* with a zone of 10mm and *Escherichia coli* with a zone of 8mm. From table 2, 3 and 4, it can be deduced that the gram negative organism (*Escherichia coli*) was more susceptible to all the extract than the gram positive (*Staphylococcus aureus*), as no zone of inhibition was observed on it. From table 2, 3 and 4 the zone of inhibition produced by the extracts on the *Escherichia coli* plate, can be compared fairly with that of the standard antibiotic (oxacilin) in Table 5 which gave the same diameter of inhibition zone to be 8mm.This observed trend might be related to the concentration of these bioactive constituents in them. Generally, the concentration of the extracts affected the rate of inhibition of growth of pathogens.

CONCLUSION

This investigation has revealed that the leaves of *Barteria nigritana* studied have high phytochemical content and have antimicrobial activity on one of the test human pathogen used in this research. This is an indication that they are of high medicinal value. Thus they could be exploited to be used in the formation of alternative antimicrobial drugs which will be used to cure and control human diseases. Apart from the antibacterial property of leaves of this plant against the pathogenic bacteria (*Escherichia coli*), this plant has an indication of high medicinal value which could also be exploited to be used in the formation of alternative antimicrobial drugs against number of human diseases due to the presence of many bioactive compounds (phytochemicals).

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